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Nitric oxide delivering surfaces: An overview of functionalization strategies and efficiency progress

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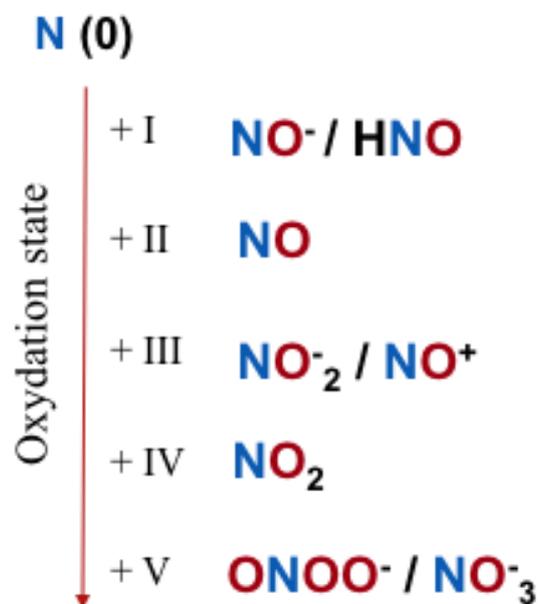
An overview on the design of nitric oxide (NO) delivering surfaces for biomedical purposes is provided, with a focus on the advances of the past 5 years. A localized supply of NO is of a particular interest due to the pleiotropic biological effects of this diatomic compound. Depending on the generated NO flux, the surface can mimic a physiological release profile to provide an activity on the vascular endothelium or an antibacterial activity. Three requirements are considered to describe the various strategies leading to a surface delivering NO. Firstly, the coating must be selected in accordance with the properties of the substrate (nature, shape, dimensions...). Secondly, the releasing and/or generating kinetics of NO should match the targeted biological application. Currently, the most promising structures are developed to provide an adaptable NO supply driven by pathophysiological needs. Finally, the biocompatibility and the stability of the surface must also be considered regarding the expected residence time of the device. A critical point of view is proposed to help readers in the design of the NO delivering surface according to its expected requirement and therapeutic purpose.

Keywords:

Nitric oxide, biomaterials, NO releasing surfaces, NO generating surfaces, endothelialization, angiogenesis, vascular tissue

## 1. Introduction

Drug release surfaces are of a particular interest in the biomedical field. They are developed to obtain a localized and controlled delivery of therapeutic molecules. The biomolecule delivery overcomes the limitations associated with the metabolism and distribution of sensitive active ingredients such as peptides or nucleic acids.<sup>[1]</sup> Among all surfaces, those that are able to release nitric oxide (NO, written NO in this article) have been extensively studied due to the pleiotropic effect of this diatomic compound. This article will emphasize on surfaces releasing and/or generating NO. This molecule is an important gasotransmitter involved in many physiological and pathological phenomena. However, its physiochemical characteristics make its use in therapy quite challenging. Indeed, it is a gaseous radical species with a short half-life<sup>[2]</sup> (on the order of several seconds depending on its location). The lifetime of NO is strongly dependent on its concentration and environment (pH, pO<sub>2</sub>, thiols, metals...) for further considerations on the physical chemistry of NO see the review of Kelm.<sup>[3]</sup> NO range of action is described to be about 100 to 200 μm from its production site.<sup>[4]</sup> NO has oxidative and/or reductive capacities leading to many essential reactions in the organism. The main by-products of NO are described in **Figure 1**. The gain of an electron leads to the nitroxyl anion NO<sup>-</sup>. In the presence of oxygen and water, NO undergoes successive oxidations leading to the formation of nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate ions (NO<sub>3</sub><sup>-</sup>). NO also reacts with the superoxide anion (O<sub>2</sub><sup>•-</sup>) to form the peroxynitrite anion ONOO<sup>-</sup>, a reactive nitrogen species responsible for nitrosative stress.<sup>[5]</sup> We refer the reader to the review written by Ford and Miranda for a more precise vision of the reactivity and fate of NO and its derivatives in physiological condition.<sup>[6]</sup>

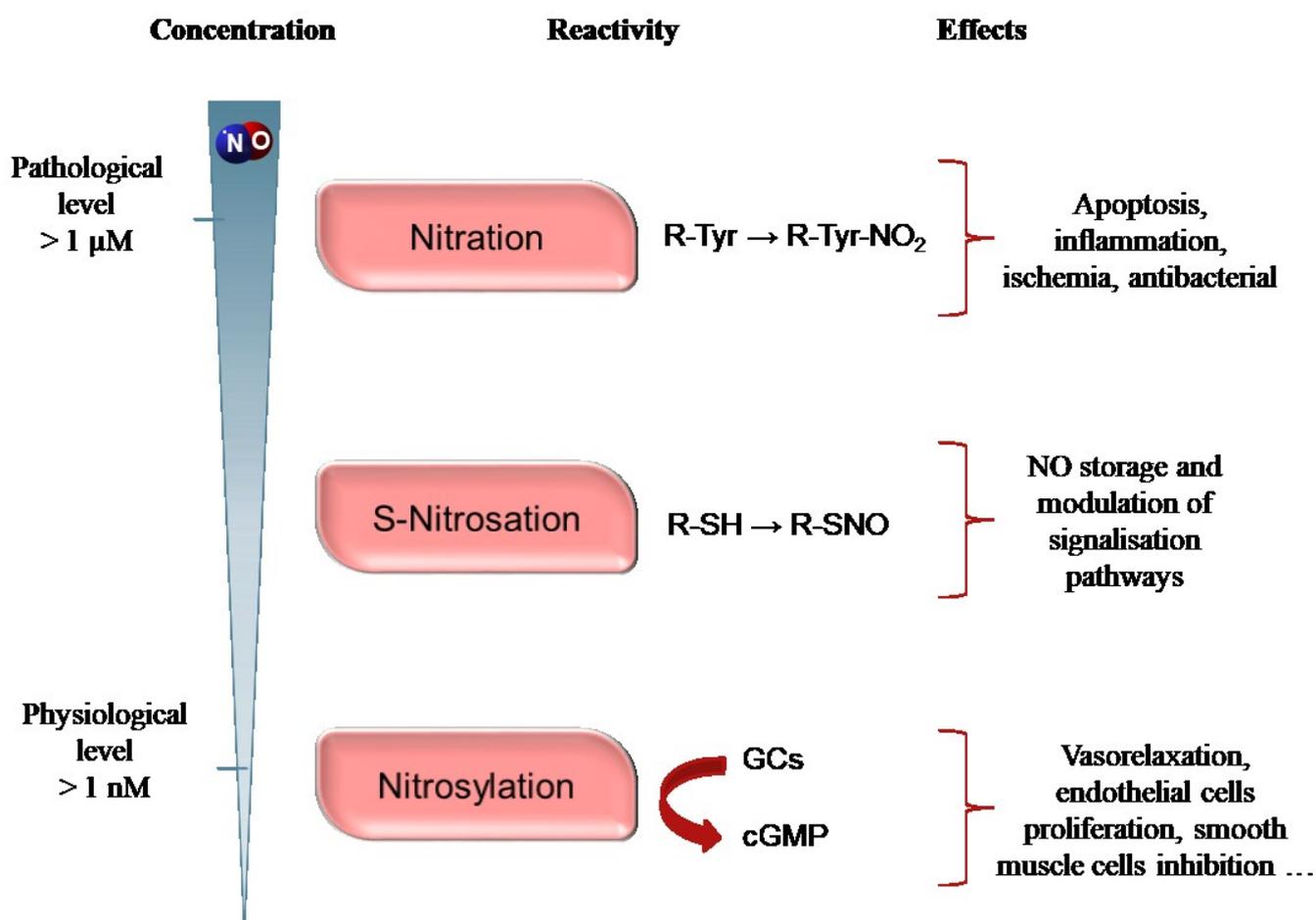


**Figure 1.** NO main byproducts obtained through successive oxidation in physiological conditions.

The reactivity and physiological effects of NO depend on its concentration and site of production (**Figure 2**). At high concentration ( $> 1 \mu\text{M}$ ) the main reaction is nitration corresponding to the addition of an  $-\text{NO}_2$  group. Classically this reaction occurs between the peroxy nitrite anion and the tyrosine residue of a protein. The formation of nitrotyrosine induces a modification of the tertiary structure of proteins leading to an alteration of their function.<sup>[7]</sup> This nitrosating species can also induce DNA damages.<sup>[8]</sup> NO antimicrobial property relies on this nitrosative and oxidative stress induction. NO action leads to the disruption of vital cellular functions or structure. Such an antimicrobial agent is particularly interesting because i) it allows the targeting of a large strains of bacteria; ii) NO is also able to diffuse through a biofilm and finally iii) its wide range of targets makes it less susceptible to resistance development.<sup>[9]</sup>

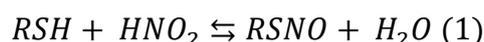
In physiological situation, at low concentrations ( $\approx \text{nM}$ ) and when produced in a regulated and transient manner, NO participates in the i) maintenance of vascular homeostasis,<sup>[10]</sup> ii) regulation of platelet aggregation,<sup>[11]</sup> iii) inhibition of monocyte adhesion,<sup>[12]</sup> iv) smooth muscle cells contraction and proliferation<sup>[13,14]</sup> and v) endothelial cells proliferation.<sup>[15]</sup> These effects are mediated by a nitrosylation corresponding to the interaction of NO with a coordination center of transition metals such as iron, copper, zinc, etc.. This interaction between NO and a metal occurs mainly in metalloprotein-dependent signaling phenomena. The majority of the physiological effects of NO comes from the interaction of NO with the heme iron of guanylate cyclase<sup>[16]</sup> which activates this enzyme and promotes the conversion of guanosine triphosphate (GTP) to guanosine monophosphate ( $\text{GMP}_c$ ). This NO- $\text{GMP}_c$  signaling pathway is of major importance by modulating the activity of several protein kinases.<sup>[17]</sup> Between these two concentrations, the NO produced by the organism mainly reacts through *S*-nitrosation. It corresponds to the reaction between a thiol functional group (RSH) and NO resulting in the formation of *S*-nitrosated species (*i.e.* *S*-nitrosothiol,

RSNO).<sup>[18,19]</sup> Thiol groups of cysteine amino-acid residues are often involved in catalytic or redox activities. A known example corresponds to the cysteine residue of endothelial NO Synthase (eNOS),<sup>[20]</sup> its *S*-nitrosation induces a negative autoregulation of NO production. Lastly, a part of the produced NO is added on low molecular weight RSNO (e.g. *S*-nitrosoglutathione, GSNO) or it can also react with proteins to form *S*-nitrosoproteins, both are the physiological forms of NO storage. GSNO is considered as the main form of intracellular NO reservoir. While, in the blood stream, NO is mainly carried by the *S*-nitrosoalbumin (AlbNO) obtained through the *S*-nitrosation of albumin free cysteine. AlbNO acts as a NO carrier over the whole blood compartment.<sup>[21]</sup>

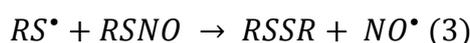


**Figure 2.** Reactions and pathophysiological effects involving NO in the organism as a function of its concentration.

Several molecular NO donors are known in the medical fields and numerous research works have been carried out to develop NO-based prodrugs for therapeutic use. However, many of them have limitations, organic and inorganic nitrate-based compounds (*e.g.* glyceryl trinitrate) do not release NO directly and need an enzymatic system to be active <sup>[22]</sup>, Sydonimines and oxadiazoles (furoxans), are known to cause high nitrosative stress *via* the production of peroxynitrite ions <sup>[23]</sup> and sodium nitroprusside cause high toxicity by the release of catalytic iron and cyanide.<sup>[24]</sup> In summary, the majority of NO donors present a fairly low bio-distribution towards their target, a tolerance phenomenon generation and a high risk of oxidative and/or nitrosative stress induction. <sup>[25]</sup> Among all these active ingredients, two families of molecules stand out. RSNO represents a judicious choice for the development of therapy to supplement NO deficiency. Indeed, beyond their capacity to bring NO to the organism, they are not toxic at physiological concentrations, do induce neither oxidative nor nitrosative stresses, nor tolerance. In the lab, they are often synthesized through the reaction between reduced thiols with nitrous acid (**Equation 1**) in water or using tert-butyl nitrite in organic solvent. <sup>[26]</sup>

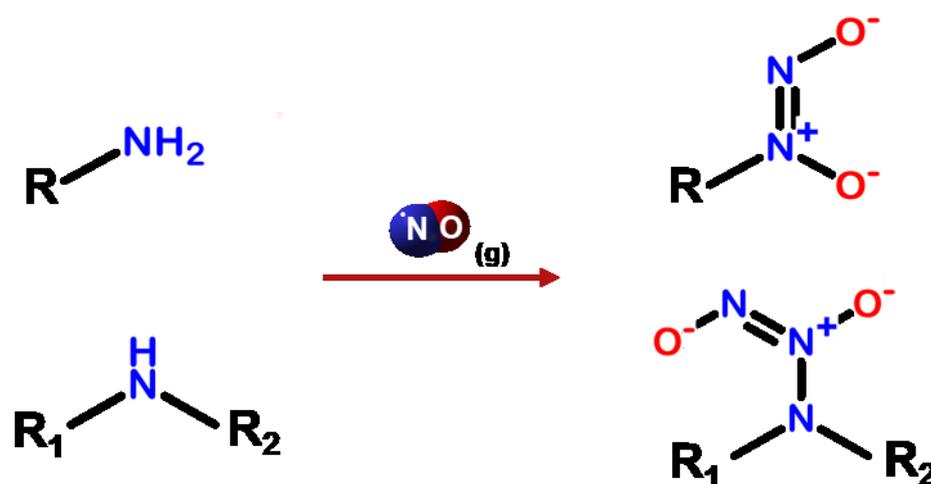


The main mechanism resulting in RSNO denitrosation is the homolysis of the S-NO bond (**Equation 2 to 4**)



This weak sulfur nitrogen bond can be cleaved by other triggers such as metallic ions (*e.g.*  $Cu^{2+}$  *via*  $Cu^+$ ), endogenous organic reducers (*e.g.* ascorbate) or enzymes (*e.g.* gamma-glutamyl transferase or protein disulfide-isomerase).

The second structures of interest are compounds containing a *N*-diazoniumdiolate function (NONOate). They are obtained by the reaction between a primary or a secondary amine with NO. NONOates have the advantage to potentially release two molecules of NO by hydrolysis of the function <sup>[27]</sup> (depending on the structure and the physicochemical conditions).

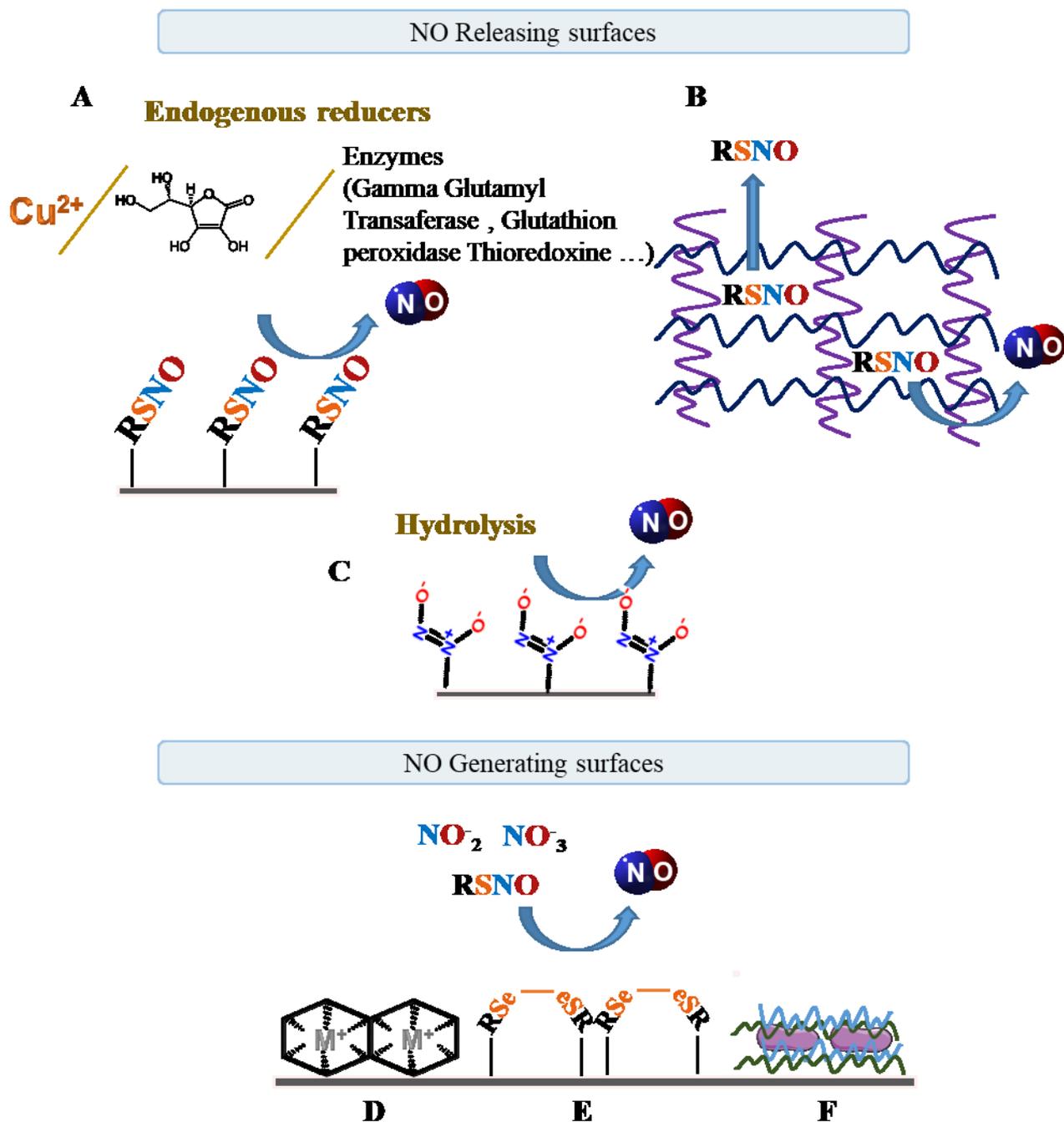


**Figure 3.** *N*-diazoniumdiolate synthesis from primary and secondary amine with gaseous NO.

NO is of great therapeutic interest, however, in its radical state it has a half-life far too short ( $\approx 1$  s) <sup>[2]</sup> to be used in this form. Although NO donors (RSNO and NONOates) have a much higher stability, they remain too labile for administration. These limitations have motivated the development of different strategies to obtain a localized NO delivery. The previously described synthesis methods of RSNO and NONOates (**Equation 1** and **Figure 3**) have been adapted in many studies to obtain surfaces functionalized by NO donors. In this way, NO releasing surfaces are materials able to store NO and release it continuously under the influence of several triggers such as thermolysis, hydrolysis or S-NO bond cleavage by physiological reducers (**Figure 4 A, B, C**). NO generating materials are defined as surfaces able to catalyze the decomposition of endogenous compounds in NO. They are generally

mimicking the activity of endogenous enzymes such as Glutathione peroxidase (Gpx). These two types of materials were created to restore or mimic a physiological release such as the one of the vascular endothelium or to confer to the surface a functionality as preventing biofilm formation. NO delivery is also of particular interest for wound healing purposes by combining antimicrobial and pro-angiogenic effects.<sup>[28]</sup>

This article was built as a progress report focused on materials and surfaces able to release or to generate NO. Here, the methods to obtain colloidal objects having the same properties will not be discussed and can be found in other review articles.<sup>[29-31]</sup> The development of functionalized surfaces imposes several constraints and limitations according to the adopted strategies. Firstly, the coating must be in adequacy with the original properties of the substrate (nature, shape, dimensions...). Secondly, a quantity of NO and a delivery kinetics compatible with the target biological effect must be achieved. Finally, the biocompatibility and the durability of the surface must also be considered. Since the identification of the physiological role of NO about thirty years ago, many works have been studying the involvement of this diatomic compound in the vascular homeostasis<sup>[10]</sup> and the immune phenomena<sup>[32]</sup>. More recently, NO and the molecules that can provide it, have been considered as potential drug candidates<sup>[33]</sup> and it is in this light that biomaterials that can generate or release NO have been imagined. Over the past five years (2016-2021) many studies have been carried out on these biomaterials and they will be discussed in the following sections.



**Figure 4.** Overall scheme of the NO release by a functionalized surface (Top part: A, B, C). (A) NO release from a covalently bound RSNO. (B) NO release and/or RSNO leaching from a liquid impregnated material. (C) NO released from a *N*-diazeniumdiolate functionalized surface. Overall scheme of the NO generation by a surface catalyzing endogenous NO source degradation (bottom part: D, E, F). NO generation from immobilized metallic reducer (D), organoselenium species (E) and enzymes (F).

## 2. NO Delivery

The study and the creation of NO delivering surfaces involve several challenges. Indeed, beyond the development of functionalization strategies, the quantity of available NO and its release kinetics are key points. As stated in the introduction, NO effect is mainly dependent on its concentration. The NO physiological production of the endothelium is about 0.05 to 0.4  $\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ .<sup>[34]</sup> Thus, an application mimicking the endothelial function requires these low levels. At the opposite, surfaces providing an antibacterial effect rely on higher NO flux (in the order of  $\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ ).

### 2.1 Materials functionalized by NONOates

Surfaces functionalized by NONOates (**Figure 4C** and **Table 1**) are obtained by the reaction between an amine and gaseous NO under a high pressure to form a *N*-diazoniumdiolate function (with sodium methoxide as a catalyst).

**Table 1.** NO releasing materials based on NONOate functionalization

NO source	Surface	NO release profile	Application	Results	Ref
<i>N</i> -diazoniumdiolate functionalized silane	Titanium implant	Burst release of $78 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$	Antibacterial	0.8 log reduction of the number adhered bacteria ( <i>P. aeruginosa</i> )	<sup>[35]</sup>
Graphene oxide multilayered film functionalized with <i>N</i> -diazoniumdiolate	Si wafers and poly(ethylene terephthalate glycol)	Total NO load of $0.3 \mu\text{M}\cdot\text{cm}^{-2}$ with releasing time of 3 days	Wound healing	Non-cytotoxic for human dermal fibroblasts. Cell migration properties and wound closure promoted by	<sup>[36]</sup>

				NO release
Heparin nanoparticles functionalized by <i>N</i> -diazoniumdiolate	Stainless steel coated with poly(glycidyl methacrylate) immobilizing particles	Maximum release of 385.2 $\mu\text{M}$ for 10 days with a burst release of 167 $\mu\text{M}$ during the first 12 h.	Synergistic effect of NO and heparin release allowed endothelialization and anticoagulant effects	Complete endothelialization of a stent after implantation in a rabbit atherosclerosis model
Poly(dopamine) coating functionalized by <i>N</i> -diazoniumdiolate	Glass substrate	Burst release within the first few hours followed with a sustained flux for 48h	Antibiofilm	Bacteria ( <i>P. aeruginosa</i> ) killing study with functionalized coating showed 99, 96 and 70 % of reduction of bacterial attachment after 6, 24 and 36 h on the surface compared to unmodified glass.
Poly(ethyleneimine) functionalized with <i>N</i> -diazoniumdiolate	Multilayered film of PEI and alginate	Maximal NO load of 0.3 $\mu\text{M}\cdot\text{cm}^{-2}$ with releasing time of 30 hours	No biological application tested	-
Poly( $\epsilon$ -lysine) functionalized with <i>N</i> -diazoniumdiolate	Contact lens gels obtained with Cross-linked hydrogel of poly( $\epsilon$ -lysine) and nonanedioic	15 hours release with a total gel load of 30 $\mu\text{M}\cdot\text{mg}^{-1}$	Microbial keratitis treatment	Bacteria killing study showed a significant proliferation reduction of <i>S. aureus</i> and <i>P. aeruginosa</i> . Corneal

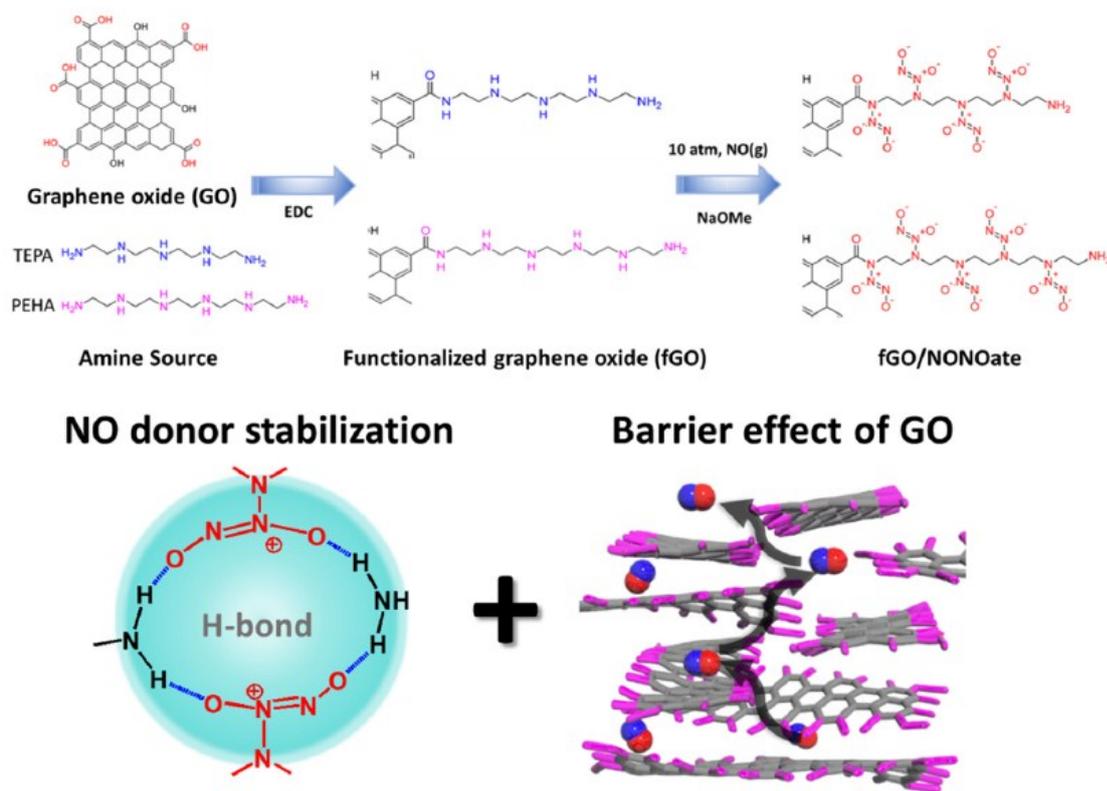
acid

epithelial cell  
metabolic  
activity  
maintained even  
with this high  
NO release

These amines can be initially present on the surface or brought *via* a synthesis strategy. *N*-diazoniumdiolate formed with a secondary amine can release two NO at physiological pH while the one obtained from a primary amine is decomposed in NO and HNO reducing the quantity of NO produced by this type of materials.<sup>[41]</sup> The primary advantage of this functionalization strategy is that the release of NO does not respond to a specific trigger allowing a continuous release. The degradation of the *N*-diazoniumdiolate function into NO is dependent on the pH and hydrolysis. The flux of NO release can also be modulated by modifying the structure or the composition of the materials. Neighboring primary amines can increase the *N*-diazoniumdiolate stability<sup>[27]</sup> (through H bound interaction) and can therefore slow down the release of NO. Polyelectrolyte multilayer (PEM) films are of a great interest in this context. They combine the benefits of their easy build up on any type of substrate and their possibility to use a polymer with high amine content suitable for functionalization and stabilization. Jeong and co-workers developed PEM coatings applied through the Layer-by-Layer (LbL) method and composed of branched poly(ethyleneimine) and alginic acid.<sup>[39]</sup> They optimized the conditions of film construction to promote the highest polymer deposition on the surfaces in order to obtain the highest NO loading. The diffusion of water and thus the hydrolysis rate (*i.e.* the NO releasing flux) can be reduced<sup>[42]</sup> by adding a hydrophobic polymeric layer such as poly(urethane) to the film. Tanum and co-workers (**Figure 5**) reported multilayered films with a sustained release of NO provided amine stabilization combined to gas barrier properties of graphene oxide (GO).<sup>[36]</sup> Graphene oxide was functionalized with tetraethylenepentamine (TEPA) or pentaethylenehexamine (PEHA).

These molecules contain several secondary amines, for NONOates formation, and as well as two primary amines at their ends allowing an amide linkage with GO through a carbodiimide coupling and also conferring a positive charge to GO. Multilayered films were built with oppositely charged GO sheets, the positive layer being the NONOate functionalized GO while the negative one corresponded to native GO. They demonstrated a significant increase of NO half-life and the total release duration depended on the number of layers highlighting the barrier effect carried by the graphene. NONOates functionalized surfaces all show the same NO release profile. They are characterized by a massive liberation within a few hours followed by a sustained release of a few days at most. It is therefore complicated to consider a long-term activity for this type of material. However, they could be well suited for antibacterial action in medical devices with low residence time (< 24h according to ISO 10993 standard). Based on this, Aveyard and co-worker created contact lens gels with a NO releasing hydrogel for the treatment of microbial keratitis.<sup>[40]</sup> The duration of NO release (15 h) is in accordance with the release time needed for this type of medical device.

In a general way, *N*-diazoniumdiolate functions are known to produce toxic by-products such as nitrosamine. These molecules can induce a nitrosative stress and they are not FDA approved, thus limiting their use at the moment in the field of biomaterials. One of the strategies used to overcome these potential toxicity issues is the functionalization of biomaterials with endogenous NO donor. Another limitation related to this type of surfaces is their ease to degrade in contact with water or heat. In this way, in order to fully consider this type of structure for a medical device, it is important to select an appropriate method for sterilization (e.g. ethylene oxide) and storage (lyophilisation). This may be a problem with the use of NONOates.



**Figure 5.** Graphene oxide functionalization by amine and NONOates. Amine stabilization of NONOate function (through H bond) and gas barrier effect carried by the LbL organization of graphene. (Reproduced from Tanum *et al.* 2019<sup>[36]</sup> with permission, 2021, Elsevier)

## 2.2 Materials functionalized by RSNO

Adding RSNO on a surface as a reservoir of NO presents several advantages. *S*-nitrosothiols are considered as the NO donors with minimal toxicity and without oxidative/nitrosative stress induction. The half-lives of RSNO, ranging from hours to several days (*e.g.* SNAP  $t_{1/2} = 1\text{h}$ , GSNO  $t_{1/2} = 40\text{h}$ )<sup>[33]</sup> are generally greater than NONOates (typically ranging from a few seconds to 24 hours).<sup>[43]</sup> The addition of RSNO in a material is mainly achieved through two strategies. The first one consists in entrapping RSNO in a matrix to obtain a material able of NO delivery through RSNO degradation and/or leaching (**Figure 4.B**). The second strategy is based on covalent anchoring of RSNO molecules or addition of a nitrosable function (thiols) to the material (**Figure 4.A**).

**Table 2.** NO releasing materials based on RSNO impregnation or RSNO covalent functionalization

	NO source	Surface	Flux	Application	Results	Ref
Liquid impregnation	GSNO	Poly(vinyl alcohol)/Poly(ethylene glycol) films containing RSNO and silver nanoparticles (AgNP)	15 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Synergistic effect of GSNO and AgNP for Antibacterial and antitumor effect	<i>In vitro</i> assay revealed a significant reduction in cancerous cell viability	[44]
	SNAP	Impregnated silicone rubber tubing	0.2 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 2 weeks	Blood compatible surface	<i>In vivo</i> rabbit extracorporeal circuit thrombosis model showed an antithrombotic effect	[45]
	SNAP	Impregnated silicone rubber cannula	Initial burst release followed by a sustainable flux Over 0.05 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 2 weeks	Antibacterial effect for insulin pump cannula	Over 90 % of reduction of <i>S. aureus</i> and <i>S. epidermidis</i> adhesion and viability during 24 h	[46]
	SNAP	Impregnated silicone catheter	Initial burst release followed	Antibacterial effect for urinary	Around 2 log reduction of <i>S.</i>	[47]

		by a sustainable flux Over 0.05 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 2 months	catheter	<i>aureus</i> and <i>P. aeruginosa</i> viability after 7 days
SNAP	Impregnated poly(dimethylsiloxane) (PDMS)	0.1 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 24 h	Blood compatible surface	75 % reduction of platelets adhesion and 80 % of <i>S. aureus</i> adhesion reduction [48]
SNAP	Spin coating of impregnated poly(urethanes) on silicone wafers	Burst release of 1.2 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 1 h followed by a flux of 0.2 nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 24 h	Anti-bacterial surface	95 % of <i>S. aureus</i> viability reduction [49]
Fluorinated SNAP	Impregnated poly(vinylidene fluoride) tubing	Increase of SNAP half-life by fluorination. NO release > 0.2	Anti-bacterial surface	Respectively 1 and 2 log reduction of <i>S. aureus</i> and <i>P. aeruginosa</i> [50]

			nmol.cm <sup>-2</sup> .min <sup>-1</sup> during 3 days and >0.05 nmol.cm <sup>-2</sup> .min <sup>-1</sup> for up to 7 days		<i>a</i> viability	
	SNAP -fibrin microparticles	Cross linked Poly(ethylene glycol) hydrogel containing functionalized microparticles	0.015 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Adherent hydrogel releasing NO for wound healing	No biological efficiency proof of concept	[51 ]
	Nitrosated thioglycolic acid (TGA)	Titania nanotubes arrays coated with multilayer film of chitosan-TGA- SNO and heparin	Release of 0.04 nmol.cm <sup>-2</sup> during 20 minutes	Blood compatib le surface	Platelet and leukocyte adhesion reduction	[52 ]
Covalentl y bound RSNO	RSNO modified mesoporous silica nanoparticles	Polyurethane membrane containing the functionalized particles	Burst release of 4.2 μmol.cm <sup>-2</sup> during 20 minutes	No biological application		[53 ]
	SNAP covalently linked to poly(dimethylsilo xane) (PDMS)	(PDMS) substrate	4.3 μmol. cm <sup>-2</sup> released in 25 days with a maximu m flux of 0.4 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Antibacte rial effect and anti- platelet adhesion	99.99 % and 88 % reduction of <i>S.</i> <i>aureus</i> and platelets adhesion	[54 ]

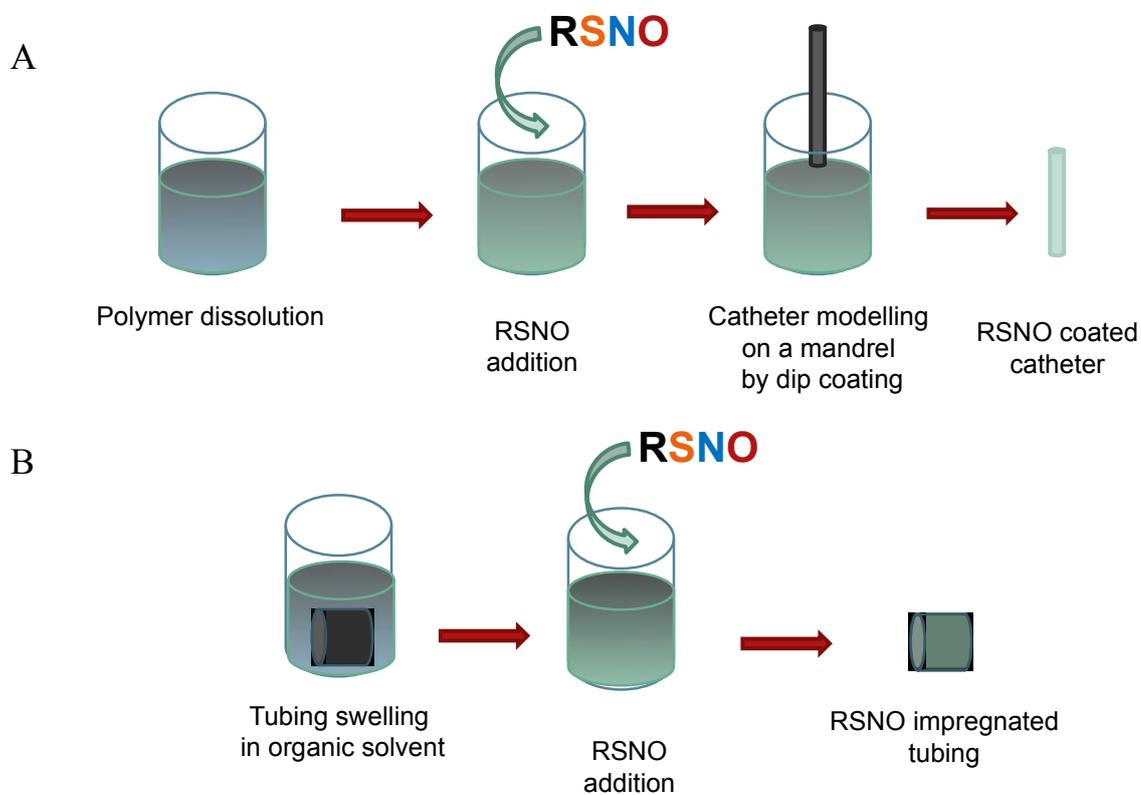
GSNO: S-Nitrosoglutathione, SNAP: S-Nitroso-N-acetylpenicillamine

### 2.2.1 Entrapped RSNO: Material impregnation with liquid RSNO

Impregnation in the case of tubular materials can be achieved by swelling a polymeric tubing in an organic solvent to increase its permeability and allow the diffusion of molecules within the material.<sup>[55]</sup> The alternative method consists in completely dissolving the tubing in an organic solvent and reforming a new material integrating RSNO (**Figure 6**). Regardless of the selected method, the solvent selection is the key point for liquid impregnation. It must induce an efficient polymer swelling without altering the tubing stability or on the contrary allow a total dissolution of polymeric tubing in order to model a new one.

As reported in the **Table 2**, *S*-nitroso-*N*-acetyl penicillamine, SNAP, is the most frequently used RSNO for the functionalization strategy based on material impregnation. Indeed, this NO donor is a suitable choice because of its positive partition coefficient (Log P = 0.96) showing its lipophilicity and good solubility in organic solvent.<sup>[33]</sup> For example, Wo et al 2017 determined that a mixture of methylethylketone and methanol (70%/30% MEK/MeOH) allowed an efficient swelling for incorporating SNAP on a poly(urethane) film without damaging the material.<sup>[56]</sup> They studied the RSNO incorporation kinetic and found a maximum SNAP impregnation after 2 hours of soaking. The efficiency of these types of materials were already studied *in vivo*. Devine and coworkers tested the thrombus formation on a rabbit model.<sup>[45]</sup> An extra corporeal circulation (ECC) model was used without and with SNAP impregnation. Continuous release of NO allowed by the addition of SNAP exhibited a significant anti-thrombogenic effect. This functionalization strategy also demonstrated an anti-bacterial activity against *S. aureus* and *S. epidermidis* when used for an insulin cannula.<sup>[46]</sup> Liquid impregnation is an effective strategy to obtain RSNO functionalized surfaces. However, it is limited to a few types of material (mainly polymeric tubing) and therefore for

quite a few therapeutic applications. A wider range of NO releasing surface coatings can be created by covalently attached RSNO on the surface



**Figure 6.** Schematic representation of the RSNO impregnated tube-making process. (A) Modeling of a RSNO impregnated catheter from a polymer solution. (B) Tubing swelling followed by a RSNO impregnation.

### 2.2.2 NO releasing surfaces functionalized by covalently bound *S*-nitrosothiols

Materials functionalized by covalently bound *S*-nitrosothiols might be considered as a promising source since these species represent the physiological storage form of NO. A few research groups have recently worked on the development of this type of surfaces. The functionalization relies on a two-step process. First, it needs the addition of a reduced thiol precursor to the surface that can be nitrosated. By following this strategy, Goudie and coworkers created a polydimethylsiloxane substrate grafted with SNAP.<sup>[54]</sup> Their optimal construction reached a NO load of 4.3  $\mu\text{mol}\cdot\text{cm}^{-2}$  released in 25 days and showed a significant antibacterial activity against *S. aureus*. Surfaces functionalized with covalently bound RSNO present significant advantages. In contrast to materials designed by liquid impregnation a wide variety of substrate can be functionalized. Unlike materials bearing NONOate, RSNO functionalized surfaces mimic an endogenous source of NO and therefore do not present a toxicity risk. Lastly, in physiological conditions, only NO is released by this type of surfaces without the need of specific triggers. However, covalently bound RSNO approach faces a major limitation in term of quantities of NO loaded. This phenomenon could be explained by the construction process of these materials. After addition of reduced thiols, they rapidly tend to the formation of disulfides thus reducing the number of functions available for nitrosation.

### 2.3 Nitric oxide formation catalyzed by the surface

The immobilization of NO on a surface regardless of the functionalization strategy employed represents a limited storage of available NO. An alternative strategy used to overcome this restriction is to generate NO from endogenous molecules. In this way, surfaces having a catalytic activity were created to potentially obtain an endless NO generating system (**Table 3**). The section below focuses only on materials, for which the reactions occurring on their surface directly produce as main product the free radical NO. In order to bring a global vision, we would like to specify that there are alternative indirect systems producing molecules that can lead to the formation of NO. The most frequently encountered materials of this type are silicon nitrides bioceramics ( $\text{Si}_3\text{N}_4$ )<sup>[57]</sup>.

Briefly, in aqueous solution, the homolytic rupture of these structures generate on the one hand reactive species of nitrogen (ammonia,  $\text{NH}_3$ ) and on the other hand reactive oxygen species (silanol, Si-O). Then, by secondary reactions within the environment, one of the possible by-products is NO<sup>[58]</sup>. These biomaterials are mostly used in the orthopedic field<sup>[59]</sup> for knee or hip joint replacement<sup>[60]</sup>. Several studies have demonstrated the ability of these silicon nitride-based materials to promote osteogenesis<sup>[61,62]</sup>, to lyse bacteria such as *Staphylococcus epidermidis*<sup>[58]</sup> or *Porphyromonas gingivalis*<sup>[63]</sup> and to inactivate viruses such as SARS-CoV-2<sup>[64]</sup>. Therefore, this type of structure could be an interesting strategy to limit the risks of bacterial infections related to the implantation of osteoarticular prosthesis. However, the production by these materials of reactive nitrogen / oxygen species, such as peroxy nitrite ions, can represent a significant risk of nitrosative and oxidative stress induction.<sup>[5]</sup> Finally, as this biomedical application does not rely solely on the generation of NO we will not develop it further in this section. We invite the readers wishing more information on the use of bioceramics in the biomedical fields to consult review articles

focused on this subject. [65,66]

**Table 3.** NO generating materials through glutathione peroxidase (Gpx) like activity based on metallic ions or organoselenium compounds.

NO source	Surface	Flux	Application	Results	Ref
Copper catechol amine network	Stainless steel (316 L SS) coated	From 7 to 75 $\text{nmol.cm}^{-2}.\text{min}^{-1}$ depending on $\text{Cu}^{2+}$ loading	Cardiovascular (In stent restenosis and thrombosis)	<i>In vivo</i> stent implantation with re-endothelialization evaluation and thrombogenicity test	[67]
Copper based on metal organic framework	Impregnation of Poly(vinyl alcohol) membranes	0.2 $\text{nmol.cm}^{-2}.\text{min}^{-1}$	No biological application tested		[68]
Copper complex and ascorbic acid reducing nitrite to Nitric oxide	Immobilized on Poly(vinyl chloride) coating	Burst release	Antibiofilm activity	Biofilm (composed of Nitrospira multiformis, Nitrospira marina, and Bacillus sp.) dispersal study through bacteria proteins quantification on the surfaces	[69]
Copper based on metal organic framework	Layer-by-Layer deposition on Hydroxylated titanium foil	From 3 to 13 $\text{nmol.cm}^{-2}.\text{min}^{-1}$ depending on layers number	Cardiovascular (In stent restenosis and thrombosis)	Smooth muscle cells and macrophage inhibition, endothelial cells proliferation, Thrombogenicity	[70]

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				ty determined <i>in vivo</i> with a rabbit arteriovenous shunt model
Red ox coupling of copper and iron complexes	Immobilized on Poly (vinyl chloride) coating	Burst release	Antibiofil m activity	Biofilm (composed of Nitrospira multiformis, Nitrospira marina, and Bacillus sp.) dispersal study through bacteria proteins quantification on the surfaces [71]
Copper ions complexation by polydopamine	Polydopamin e coating on Stainless Steel (316 L SS)	0.4 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Cardiovasc ular (In stent restenosis)	Endothelial cells adhesion and proliferation enhancement and vascular remodeling after <i>in vivo</i> stent implantation [72]
Copper ions complexation polydopamine	Polydopamin e coating on Stainless Steel (316 L SS)	From 0 to 2.8 nmol.cm <sup>-2</sup> .min <sup>-1</sup> depending on Cu <sup>2+</sup> loading	Cardiovasc ular (In stent restenosis and thrombosis )	Smooth muscles cell migration and proliferation inhibition, improved reendothelializ ation through endothelial cell migration and proliferation promotion. Thrombus [73]

				formation and vessel occlusion reduction on an <i>ex vivo</i> extracorporeal circulation system.
Co-immobilization of copper phenolic amine network and selenocystamine	Gallic acid forming a polyphenolic coating compatible with various substrates: stainless steel, glass, polyethylene terephthalate, TiO <sub>2</sub>	From 0.9 to 1.4 nmol.cm <sup>-2</sup> .min <sup>-1</sup> depending on Cu <sup>2+</sup> loading	Cardiovascular	Platelets adhesion, thrombus formation and vessel occlusion reduction on an <i>ex vivo</i> extracorporeal circulation system.re-endothelialization on enhancement after in vivo stent implantation. [74]
D- or L-selenocystine	Immobilization on TiO <sub>2</sub> films with polydopamine	Higher for L-selenocystine than D-selenocystine	Cardiovascular	NO generation and platelet adhesion and activation were dependent of the selenocystine chirality [75]
Organoselenide compounds (2,2'-diselenodihydroxyethanol)	Entrapped in a polyurethane film	Stable flux after 11 days ranging from 0.04 to 0.5 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Cardiovascular	Hemocompatibility test: platelets adhesion, hemolysis, cytotoxicity. [76]

		depending on selenium loading on the film		
Organoselenium covalently linked to polyethyleneimine	Vascular graft composed of polycaprolactone fibers coated with SePEI	Flux not reported	Cardiovascular	Endothelial cells adhesion and proliferation on the surface and anti-inflammatory phenotype transformation of macrophages after <i>in vivo</i> implantation of grafts. [77]
Selenocystamine covalently linked to modified polyurethane	Polyurethane film modified by copolymerization with hydrophilic polymers	0.08 nmol.cm <sup>-2</sup> .min <sup>-1</sup>	Cardiovascular	Inhibition of platelet adhesion and smooth muscle cells adhesion and proliferation. Promotion of endothelial cell proliferation [78]

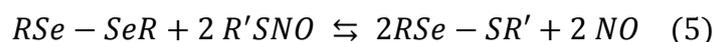
### 2.3.1 NO generation mediated by metals

Metallic ions such as copper or zinc are known to catalyze the formation of NO from endogenous NO source such as RSNO (**Figure 4D**). Several strategies are available to incorporate metal ions into a material (**Table 3**). The mostly frequent one is the formation of coordination complexes between an organic compound and a metal. Song and coworkers developed a polydopamine (DA) coating complexing Cu<sup>2+</sup> ions (Cu<sup>II</sup>-DA complexes).<sup>[73]</sup> They demonstrated that the final quantity of coordination complexes and NO generation flux

were dependent on the initial  $\text{Cu}^{2+}$  loading concentration used during the polydopamine coating construction, suggesting that the catalytic ability could be controlled. Catalytic activity of the  $\text{Cu}^{\text{II}}$ -DA coating was found stable over 15 days by maintaining a flux representing 40% of the initial NO generation. Finally, this strategy was found effective for a cardiovascular application. Coated 316L stainless steel revealed a significant ability to enhance endothelial cell growth and to reduce smooth muscle cells proliferation demonstrating the suitability of this coating to prevent in stent restenosis. NO generation mediated by the immobilization of metallic ions was also effective for biofilm inhibition purpose. Wonoputri and co-workers developed a coating containing coordination complexes of copper and iron.<sup>[71]</sup> The co-immobilization of this mixture of metallic ions created a redox cycle reducing the copper (II) to copper (I). The copper (I) formation here, was of a particular interest because of its ability to reduce nitrite ions produced by bacteria to generate NO. Another approach using the Gpx like activity of immobilized metallic ions is based on the formation of metal organic frameworks (MOF) on a surface.<sup>[79]</sup> This mesh arrangement allows the formation of wider structure with a 2 or 3-dimensional organization. Copper based MOF were created on titanium substrate by Zhao and coworkers in order to improve cardiovascular stents efficiency.<sup>[70]</sup> They employed carboxylated benzenic compounds bridging  $\text{Cu}^{2+}$  ions, this organization allowed the formation a LbL coating. The flux generated by the surface was found proportional to the number of layers. The optimal construction in term of biological efficiency was obtained with 10 bilayers. Beyond the *in vitro* effects on endothelial cells proliferation, on smooth muscle cells growth inhibition, and on platelets and on macrophages adhesion and activation, coated titanium wires effectively inhibited neointimal hyperplasia after *in vitro* implantation in rat abdominal aorta.

### 2.3.2 NO generation mediated by selenium derivate

Organoselenium species (RSe) are one of the catalysts that can generate NO. They are known to carry a Gpx-like activity.<sup>[80]</sup> The diselenide form can catalyze the denitrosation of RSNO to produce NO (**Figure 4E and Equation 5**) through redox reaction leading to the formation of selenosulfide (RSe-SR')<sup>[81]</sup>.



The active form of diselenide is regenerated (**Equation 6**) by redox reaction with reduced glutathione (GSH)<sup>[82]</sup>



Recent advances focused on the enhancement of the catalytic stability of the selenium based NO generating surfaces (**Table 3**). Qu and coworkers created a polyurethane (PU) film containing a covalently linked selenocystamine inducing a physiological level of NO.<sup>[83]</sup> They observed a continuous decomposition of RSNO for at least 30 days in a PBS/GSH solution. The kinetics of NO generation by this type of material is generally composed of two phases. The first one corresponds to a strong flux of generated NO, followed by a strong decrease leading to a plateau with a low flux. This first step corresponds to a phenomenon of material reorganization that determines the durability of the catalytic surface. The second phase is characterized by the appearance of a stable flux. In this way, Qu and coworkers reported that during the reorganization, low molecular Se-PU segments could leak from the surface while high molecular Se-PU segments are kept trapped in the PU film. Fan and coll. also studied the influence of the surface structuration on NO generation.<sup>[75]</sup> They demonstrated that the selection of the chirality of D- or L-selenocystine can significantly influence the NO generation and therefore the surface biocompatibility (*e.g* platelet

adhesion). Thus, the design of the surface in terms of structuration and composition is a key point to manage in order to keep a long-term catalytic activity. Advances are also oriented towards the construction of multifunctional platforms designed to reinforce the action of NO. Jin and coworkers created a polyvalent surface mimicking the vascular endothelium by the co-immobilization of complexes made of cyclodextrin (CD) functionalized through host-guest interaction with lysine (CD-L) and sulfonate (CD-S) on a modified polyurethane film containing selenocystamine.<sup>[78]</sup> The polyurethane substrate was modified by the copolymerization with a hydrophilic monomer (2-hydroxyethyl methacrylate (HEMA)) conferring a resistance to cell adhesion on the surface.<sup>[84]</sup> CD-L was used to add a fibrinolytic activity to the surface by adsorbing plasminogen and so on, enhance the anticoagulant effect of NO. CD-S acted synergistically with NO to promote endothelial cell proliferation by providing a heparin-like<sup>[85]</sup> function to the film. Tang and coworkers created a vascular graft composed of poly(caprolactone) fibers combining a bioinert and a bioactive strategy to enhance its blood compatibility.<sup>[77]</sup> They coated the graft with a multilayered film based on an organoselenium functionalized polyethylene imine (SePEI) and hyaluronate. They added an adhesion peptide coming from a sequence in laminin (Tyr-Ile-Gly-Ser-Arg peptide) grafted to PEG chain as the last layer of their vascular graft. *In vivo*, this organization showed an enhanced endothelialization after implantation in the abdominal aorta of rats. Interestingly, the authors also reported the infiltration of macrophages on the graft. Immunostaining of the infiltrating cells revealed the presence of M2 anti-inflammatory macrophages expressing the M2 specific marker CD 206, highlighting an increase of the anti-inflammatory phenotype induced by the *in situ* NO generation.

### 2.3.3 NO generation mediated by enzyme

The generation of NO by a surface can be achieved by the immobilization of an enzyme having for substrate a compound that can produce NO (**Figure 4F** and **Table 4**).

**Table 4.** NO generating materials based on enzymatic activity.

Surface organization	Enzyme	substrate	NO Flux	Application	Results	Ref
Silanized 316 stainless steel	Inducible NO Synthase (iNOS) covalently linked to silane	L-arginine and NADPH as cofactor	NO flux was assimilated to the enzyme activity. It was found stable and over $0.1 \text{ nmol.cm}^{-2} \cdot \text{min}^{-1}$ during 6 months	Cardiovascular	Enhanced Endothelial cells adhesion and platelet resistance compared to bare surface	[86]
Round metallic wires (alloy 35N LT, 316L and L605) coated	Beta-galactosidase immobilized on a poly(styrene sulfonate) and poly(allylamine) (PSS-PAH) multilayered film	Beta-gal-NONOate used as NO prodrug	NO flux was found dependent of the concentration of enzyme substrate (Beta-gal-NONOate). A linear flux of $0.1 \text{ nmol.cm}^{-2} \cdot \text{min}^{-1}$ was measured in presence of $20 \mu\text{M}$ of Beta-gal-NONOate	Cardiovascular	<i>Ex vivo</i> measurement of relaxation degree of rat mesenteric arteries showed a significant effect carried by the catalytic multilayered polyelectrolyte coating	[87]
	Inducible NO Synthase Oxygenase (iNOS)	L-arginine and NADPH as	linear flux of $0.3 \text{ nmol.cm}^{-2} \cdot \text{min}^{-1}$	Design of an antithrombotic coating for blood contacting	No biological evidence of the effectiveness	[88]

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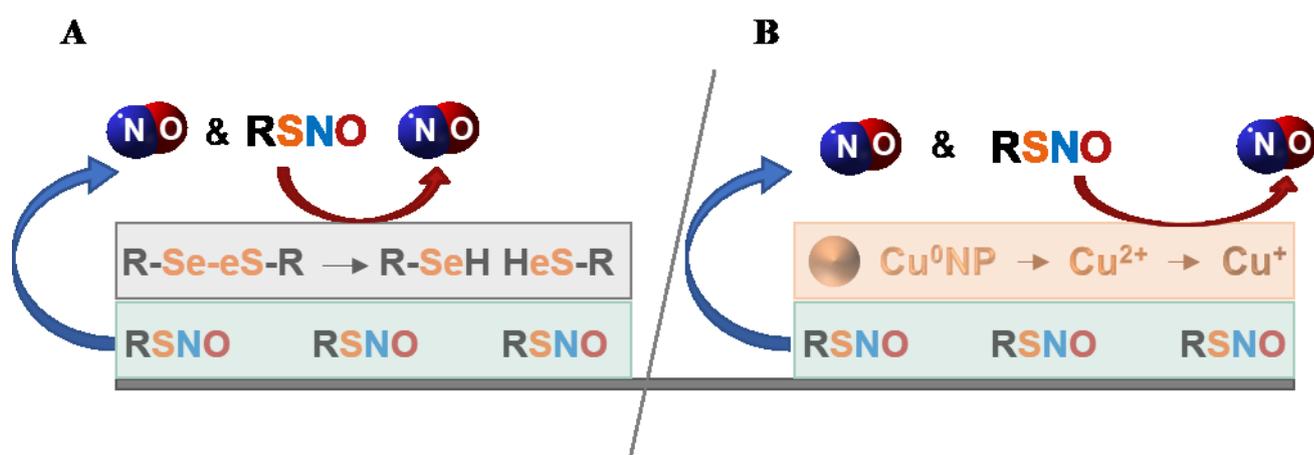
immobilized via electrostatic adsorption on Poly ethylene imine film	cofactor	medical devices	effectiveness of the biological effectiveness of NO generation by this strategy
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NO is physiologically formed by a family of enzymes called NO Synthase (NOS) by catalyzing the oxidation of L-arginine to L-citrulline with NADPH as cofactor.<sup>[89]</sup> Three types of NOS are found, neuronal and endothelial types (nNOS / eNOS) are constitutively expressed and they produce nanomolar amount of NO during short periods.<sup>[90,91]</sup> The third form of NOS is the inducible NO Synthase (iNOS) produced only in response to specific triggers such as pro-inflammatory signals. This enzyme is able to produce a greater amount of NO over a longer period of time than the other two. The iNOS was therefore considered as the most attractive to create a NO generating surface through enzyme immobilization. Alagem-Shafir and co-workers developed a coating immobilizing iNOS on stainless steel for a stent optimization purpose.<sup>[86]</sup> Stainless steel surfaces were oxidized by phosphoric acid and then silanized, iNOS was covalently linked to silanes using glutaraldehyde. Enzyme activity was maintained after linking to the surface, NO generation was indirectly determined by the quantification of the L-citrulline co-produced by the enzyme reaction after addition of L-arginine. This strategy produced a stable NO flux of  $0.1 \text{ nmol.cm}^{-2}.\text{min}^{-1}$  during 6 months and was found efficient to avoid platelet adsorption compared to a bare stainless-steel surface. Gunasekera and coworkers reached a higher NO flux of  $0.3 \text{ nmol.cm}^{-2}.\text{min}^{-1}$  by optimizing the quantity of iNOS immobilized.<sup>[88]</sup> It is interesting to notice that this value is close to the physiological level of NO production by human endothelial cells ( $0.4 \text{ nmol.cm}^{-2}.\text{min}^{-1}$ ).<sup>[92]</sup> They studied a LbL organization allowing the immobilization of iNOS through electrostatic interactions with a cationic polyelectrolyte (poly(ethyleneimine)). By modifying the pH of the medium during the film construction and therefore the net charge of the protein, they

observed a higher protein immobilization on the PEM film. Even if the use of iNOS as a NO catalyzer seems interesting, the long-term effectiveness of this type of coating is under debate. Indeed, iNOS is known to follow a self-inhibition mechanism regulated by its own NO production. In order to avoid this type of limitation due to the enzymatic regulation phenomena, Winther and coworkers developed an enzyme-prodrug strategy without using an enzyme regulated by NO.<sup>[87]</sup> They created a catalytic surface by immobilizing  $\beta$ -galactosidase on LbL films composed of poly(allylamine) (PAH) and poly(styrene sulfonate) (PSS). NO production was initiated by the addition of a functionalized substrate, the  $\beta$ -galactoside-NONOates, which can be hydrolyzed by the surface. NO flux was found dependent of the concentration of enzyme substrate ( $\beta$ -gal-NONOate) and a linear flux of  $0.1 \text{ nmol.cm}^{-2}.\text{min}^{-1}$  was measured in presence of  $20 \text{ }\mu\text{M}$  of  $\beta$ -gal-NONOate.

#### 2.4 Hybrid materials: NO release and generation

Hybrid materials are materials combining both strategies of NO release and NO generation previously described (Table 5). These materials represent surfaces containing a NO donor and a catalyzer (Figure 7).



**Figure 7.** Hybrid materials providing NO release or NO generation from leached and endogenous RSNO catalyzed by coatings based on an organoselenium (A) or containing metallic species (B).

**Table 5.** NO release mediated by hybrid materials

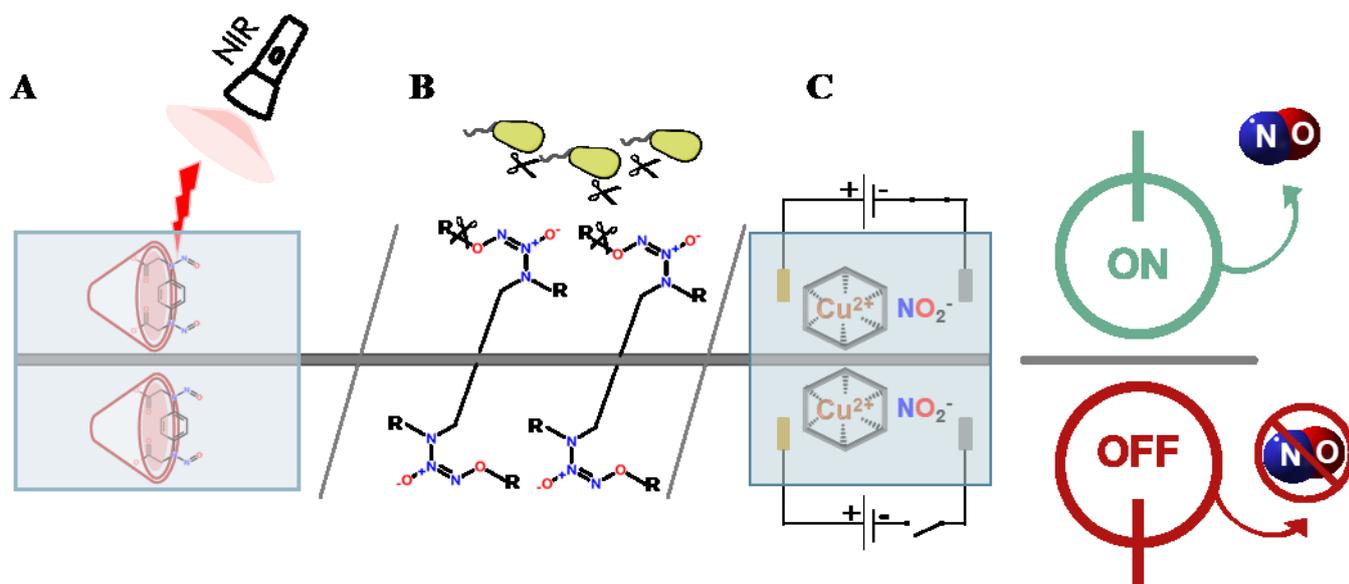
Surface organization	NO storage	NO releasing strategy	NO Flux	Application	Results	Ref
Medical grade polymer incorporating RSNO and reducer	SNAP	Copper nanoparticles	From 0.1 to 0.4 nmol.cm <sup>-2</sup> .min <sup>-1</sup> depending on CuNP loading	Antibacterial and anti-platelet adhesion	Up to 5 log reduction of the amount of forming bacterial (gram negative and positive) unit on the SNAP/CuNP doped polymer	[93]
Medical grade polymer incorporating RSNO and reducer	SNAP	Zinc oxide nanoparticles		Antibacterial		[94]
SNAP/organoselenium on a Polyurethane coating	Liquid impregnation with SNAP	organoselenium coating		Anti-thrombus and bacterial		[95]

SNAP: S-Nitroso-N-acetylpenicillamine, CuNP : Copper Nanoparticles

The selection of the quantity of the two compounds allows the modulation of the NO release from the surface. In addition, once the NO stock has been completely consumed, the catalyzer in the material can still act as a NO generating surface. A physiological flux allowing a cardio-vascular application was reached thanks to this strategy.

### 3. Smart materials: inducible release

The optimal material should be able to deliver NO only when pathophysiological conditions require it. Based on this principle, several teams created inducible systems responding to specific triggers (**Figure 8 and Table 6**).



**Figure 8.** Smart material releasing NO under a specific trigger, (A) Hydrogel containing  $\beta$ -CD functionalized with a photolabile *N*-dinitroso donor triggered by Near Infra-Red (NIR). (B) Polymer composite embedding protected NONOate responding to bacterial nitro reductase. (C) NO emitting electrochemical device enabling the reduction of nitrite by Cu(II) complexes.

**Table 6.** Surfaces with an inducible NO release.

Surface organization	NO storage	NO releasing strategy	NO Flux	Application	Results	Ref
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Crosslinked hydrogel of Hyaluronic acid and poly(dopamine)	B-cyclodextrin functionalized with a photolabile <i>N</i> -dinitroso donor embedded in the hydrogel	NIR photolysis of the <i>N</i> -dinitroso function		Bacteria-infected wound healing	<i>In vivo</i> efficiency of the hydrogels for wound healing revealed a higher inflammatory response followed by an enhancement of re-epithelialization and angiogenesis [96]
Poly(vinyl chloride) / Poly(urethane) film containing NONOate	NONOate function protected by a nitroaromatic group	NONOate function deprotection by bacterial enzymes	2.7 nmol. cm <sup>-2</sup> .min <sup>-1</sup> with a total release of 4 μmol	Antibacterial effect against bacteria expressing nitroreductase enzymes	Bacteria killing assay revealed 60 % cell viability with <i>E. coli</i> [97]
Silicone rubber patch	Copper (II) coordination complexes and nitrites	Electrochemical NO release induced by nitrite reduction by copper	Between 0.08 – 0.10 nmol. cm <sup>-2</sup> .min <sup>-1</sup>	Antibacterial	Over 99% reduction of cell viability with <i>E. coli</i> and <i>S. aureus</i> [98]

Lee and coworkers developed patch emitting NO by an electrochemical modulation.<sup>[98]</sup> An inducible system was created by incorporating heterogenic coordination complexes formed with copper and nitrite ions interacting with tri(2-pyridylmethyl)amine. The nitrite reduction to NO is managed by the application of a low voltage. This strategy was found effective to inhibit the bacterial proliferation promoting the wound healing. Hibbard and coworkers developed a *N*-diazoniumdiolate function protection to stabilize this function and limit the release of NO.<sup>[97]</sup> This NONOate prodrug was entrapped in a PU/PVC medical grade polymeric film. The deprotection reaction is induced by a bacterial nitroreductase enzyme frequently expressed by pathogenic bacteria such as *E. coli*. The release of NO is initiated

only in the presence of nitrate reductase producing bacteria and the flow of NO is proportional to the quantity of bacteria. This organization also showed the limitation of the NONOate strategy. To avoid unspecific release caused by the NONOate spontaneous hydrolysis, the prodrug was immobilized on a hydrophobic film. However, this type of coating has also the disadvantage of limiting the diffusion of bacterial enzymes within it. This mechanism was confirmed by the experiments conducted by this team. The increase in the amount of NONOate in the film was not correlated with an increase in the NO released. The hypothesis suggested by the authors relies on the fact that the bacterial enzymes only degrade the active ingredients that are available on top of the film.

#### 4. Conclusions and outlooks

In this article, we described the different strategies to obtain surfaces presenting localized release and/or generation of NO, they all have same advantages and limitations which conditions their application (**Table 7**).

**Table 7.** Summary of characteristics, advantages, limitations and applications of NO releasing and generating strategies.

Strategies	NO source	Disadvantages	Advantages	Recommended (optimal) application
		-Burst release		
NO releasing	NONOates	-Potential toxicity (nitrosamine) -Stability	high quantities immobilized	Short time antibacterial effects

	RSNO impregnation	-Very limited compatible materials	Ease of creation	Reduction of the risks of infection and thrombosis for blood contacting polymeric cannulas
	Covalently bound RSNO	- Rarely high quantities immobilized -Stability	-Wide variety of functionalizable surfaces -Limited risk of toxicity	Low efficacy currently for being applicable
NO generating	Organoselenium	-Ineffective in cases of physiological RSNO deficiency	-Limited risk of toxicity -Almost endless NO generation	Suitable for mimicking a physiological release activity such as vascular endothelium
	Metallic reducers	-Potential toxicity (metallic ion leaching) - Ineffective in cases of physiological RSNO deficiency	-Almost endless NO generation	Suitable for mimicking a physiological release activity such as vascular endothelium
	Enzyme	-Possible enzyme inhibition or degradation - NO flux depending on the enzyme substrate quantity	NO flux depending on the enzyme substrate quantity	Application is determined by the surface design
	Hybrid materials	NO Store + Reducer -Has the drawbacks of the selected strategies to store NO and to generate it	-Tunable flux according to the proportions between NO stored and reducer - Can still act as a NO generating material once the	Antibacterial effect if designed for a burst release of the stored NO

NO stored is  
consumed

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Smart materials	Several possible approaches	Complex design	-Inducible release -Flux proportional to the pathophysiological need	Application is determined by the surface design
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The materials based on NONOates functionalization are very sensitive to hydrolysis. This leads to a burst release under physiological condition, or it imposes the design of very hydrophobic coatings. They also present a risk of production of toxic by-products (nitrosamine)<sup>[99]</sup>. Therefore, it is preferable to reserve their use to create materials with a short time antibacterial effect and with a short time of contact. RSNO liquid impregnation is a strategy allowing the creation of high NO loaded materials with a sustainable release. However, it is only compatible with plastic polymer matrices. Thus, they are highly effective in preventing biofilm and thrombus formation on the inner face of cannulas dedicated to extracorporeal blood circulation. The functionalization of materials with covalently bonded RSNO is probably the most challenging strategy. High NO load is problematic to achieve, probably because the thiols and RSNO functions are highly redox-sensitive. But, this strategy remains interesting to be developed because it is mimicking the physiological form of NO storage and thus does not present a toxicity risk. The effectiveness of NO generating surfaces could be limited in case of deficiency in endogenous NO sources frequently occurring in pathophysiological situation. In addition, materials releasing metal ions may present toxicity issues and enzyme-based surfaces could encounter enzymatic inhibition phenomena. Hybrid materials result from the addition of a NO release and NO generation strategy. They have the limitations and constraints of the strategies they combine. Thus, the surfaces immobilizing

RSNO by liquid impregnation for the NO load part are limited to a restricted number of matrices and moreover the materials using metal ions for the catalytic part may also encounter toxicity issues related to the ions leaching. Smart material with an inducible NO release represents the most promising strategy. However, the complexity of their design is a limitation imposing extensive development. To conclude, to date, there is no ideal NO-delivering surface. However, we hope that this article will help readers to select the design of the NO delivering surface according to the expected final requirements (NO load, flux, release time, location...).

### Conflict of interest

The authors declare no conflict of interest.

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### References

- [1] A. N. Zelikin, *ACS Nano* **2010**, *4*, 2494.
- [2] D. S. Bredt, S. H. Snyder, *Annu. Rev. Biochem.* **1994**, *63*, 175.
- [3] M. Kelm, *Biochim. Biophys. Acta Bioenerg.* **1999**, *1411*, 273.
- [4] J. R. Lancaster, *Nitric Oxide* **1997**, *1*, 18.
- [5] J. R. Lancaster, *Chem. Res. Toxicol.* **2006**, *19*, 1160.
- [6] P. C. Ford, K. M. Miranda, *Nitric Oxide* **2020**, *103*, 31.
- [7] M. L. Jones, J. G. Ganopoulos, A. Labbé, C. Wahl, S. Prakash, *Appl. Microbiol. Biotechnol.* **2010**, *88*, 401.
- [8] M. J. Juedes, G. N. Wogan, *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **1996**, *349*, 51.
- [9] M. A. D. Groote, F. C. Fang, *Clin. Infect. Dis.* **1995**, *21*, S162.

- [10] J. Loscalzo, G. Welch, *Prog. Cardiovasc. Dis.* **1995**, *38*, 87.
- [11] M. E. Mendelsohn, S. O'Neill, D. George, J. Loscalzo, *J. Biol. Chem.* **1990**, *265*, 19028.
- [12] P. Kubes, M. Suzuki, D. N. Granger, *Proc. Natl. Acad. Sci.* **1991**, *88*, 4651.
- [13] D. S. Marks, J. A. Vita, J. D. Folts, J. F. Keaney, G. N. Welch, J. Loscalzo, *J. Clin. Invest.* **1995**, *96*, 2630.
- [14] U. C. Garg, A. Hassid, *J. Clin. Invest.* **1989**, *83*, 1774.
- [15] K. Fukuo, T. Inoue, S. Morimoto, T. Nakahashi, O. Yasuda, S. Kitano, R. Sasada, T. Ogihara, *J. Clin. Invest.* **1995**, *95*, 669.
- [16] K. A. Broniowska, N. Hogg, *Antioxid. Redox Signaling* **2012**, *17*, 969.
- [17] A. Friebe, D. Koesling, *Circ.Res.* **2003**, *93*, 96.
- [18] N. Hogg, R. J. Singh, B. Kalyanaraman, *FEBS Lett.* **1996**, *382*, 223.
- [19] A. Keszler, Y. Zhang, N. Hogg, *Free Radic. Biol. Med.* **2010**, *48*, 55.
- [20] K. Ravi, L. A. Brennan, S. Levic, P. A. Ross, S. M. Black, *Proc. Natl. Acad. Sci.* **2004**, *101*, 2619.
- [21] T. Rassaf, M. Feelisch, M. Kelm, *Free Radical Biology and Medicine* **2004**, *36*, 413.
- [22] G. R. J. Thatcher, A. C. Nicolescu, B. M. Bennett, V. Toader, *Free Radic. Biol. Med.* **2004**, *37*, 1122.
- [23] Y. C. Hou, A. Janczuk, P. G. Wang, *Curr Pharm Des* **1999**, *5*, 417.
- [24] H. Schröder, *Mol Pharmacol* **2006**, *69*, 1507.
- [25] L. J. Ignarro, C. Napoli, J. Loscalzo, *Circ. Res* **2002**, *90*, 21.
- [26] K. Szaciłowski, Z. Stasicka, *Prog. React. Kinet. Mech.* **2001**, *26*, 1.
- [27] L. K. Keefer, *ACS Chem. Biol.* **2011**, *6*, 1147.
- [28] M. C. Jen, M. C. Serrano, R. van Lith, G. A. Ameer, *Adv. Funct. Mater.* **2012**, *22*, 239.
- [29] Y. Yang, P. K. Qi, Z. L. Yang, N. Huang, *Biosurf. Biotribol.* **2015**, *1*, 177.
- [30] A. W. Carpenter, M. H. Schoenfish, *Chem. Soc. Rev.* **2012**, *41*, 3742.
- [31] N. Naghavi, A. de Mel, O. S. Alavijeh, B. G. Cousins, A. M. Seifalian, *Small* **2013**, *9*, 22.
- [32] B. Zdzisińska, M. Kandefer-Szerszeń, *Postepy Hig Med Dosw* **1998**, *52*, 621.

- [33] C. Gaucher, A. Boudier, F. Dahboul, M. Parent, P. Leroy, *Curr. Pharm. Des.* **2012**, *19*, 458.
- [34] A. de Mel, F. Murad, A. M. Seifalian, *Chem. Rev.* **2011**, *111*, 5742.
- [35] M. Li, J. Aveyard, G. Fleming, J. M. Curran, F. McBride, R. Raval, R. A. D'Sa, *ACS Appl. Mater. Interfaces* **2020**, *12*, 22433.
- [36] J. Tanum, H. Jeong, J. Heo, M. Choi, K. Park, J. Hong, *Appl. Surf. Sci.* **2019**, *486*, 452.
- [37] T. Zhu, M. Zhou, W. Gao, D. Fang, Z. Liu, G. Wu, M. Wan, C. Mao, J. Shen, *Langmuir* **2020**, *36*, 2901.
- [38] Z. Sadrearhami, F. N. Shafiee, K. K. K. Ho, N. Kumar, M. Krasowska, A. Blencowe, E. H. H. Wong, C. Boyer, *ACS Appl. Mater. Interfaces* **2019**, *11*, 7320.
- [39] H. Jeong, K. Park, J.-C. Yoo, J. Hong, *RSC Adv.* **2018**, *8*, 38792.
- [40] J. Aveyard, R. C. Deller, R. Lace, R. L. Williams, S. B. Kaye, K. N. Kolegraff, J. M. Curran, R. A. D'Sa, *ACS Appl. Mater. Interfaces* **2019**, *11*, 37491.
- [41] D. J. Salmon, C. L. Torres de Holding, L. Thomas, K. V. Peterson, G. P. Goodman, J. E. Saavedra, A. Srinivasan, K. M. Davies, L. K. Keefer, K. M. Miranda, *Inorg. Chem.* **2011**, *50*, 3262.
- [42] R. J. Soto, E. P. Merricks, D. A. Bellinger, T. C. Nichols, M. H. Schoenfish, *Biomaterials* **2018**, *157*, 76.
- [43] P. C. Ford, K. M. Miranda, *Nitric Oxide* **2020**, *103*, 31.
- [44] W. R. Rolim, J. C. Pieretti, D. L. S. Renó, B. A. Lima, M. H. M. Nascimento, F. N. Ambrosio, C. B. Lombello, M. Brocchi, A. C. S. de Souza, A. B. Seabra, *ACS Appl. Mater. Interfaces* **2019**, *11*, 6589.
- [45] R. Devine, M. J. Goudie, P. Singha, C. Schmiedt, M. Douglass, E. J. Brisbois, H. Handa, *ACS Appl. Mater. Interfaces* **2020**, *12*, 20158.
- [46] M. K. Chug, C. Feit, E. J. Brisbois, *ACS Appl. Bio Mater.* **2019**, *2*, 5965.
- [47] K. H. Homeyer, M. J. Goudie, P. Singha, H. Handa, *ACS Biomater. Sci. Eng.* **2019**, *5*, 2021.
- [48] R. Devine, P. Singha, H. Handa, *Biomater. Sci.* **2019**, *7*, 3438.
- [49] P. Singha, J. Pant, M. J. Goudie, C. D. Workman, H. Handa, *Biomater. Sci.* **2017**, *5*, 1246.
- [50] Y. Zhou, Q. Zhang, J. Wu, C. Xi, M. E. Meyerhoff, *J. Mater. Chem. B* **2018**, *6*, 6142.

- [51] C. A. Joseph, C. W. McCarthy, A. G. Tyo, K. R. Hubbard, H. C. Fisher, J. A. Altscheffel, W. He, R. Pinnaratip, Y. Liu, B. P. Lee, et al., *ACS Biomater. Sci. Eng.* **2019**, *5*, 959.
- [52] R. Simon-Walker, R. Romero, J. M. Staver, Y. Zang, M. M. Reynolds, K. C. Popat, M. J. Kipper, *ACS Biomater. Sci. Eng.* **2017**, *3*, 68.
- [53] M. J. Malone-Povolny, M. H. Schoenfisch, *ACS Appl. Mater. Interfaces* **2019**, *11*, 12216.
- [54] M. J. Goudie, P. Singha, S. P. Hopkins, E. J. Brisbois, H. Handa, *ACS Appl. Mater. Interfaces* **2019**, *11*, 4523.
- [55] N. MacCallum, C. Howell, P. Kim, D. Sun, R. Friedlander, J. Ranisau, O. Ahanotu, J. J. Lin, A. Vena, B. Hatton, et al., *ACS Biomater. Sci. Eng.* **2015**, *1*, 43.
- [56] Y. Wo, Z. Li, E. J. Brisbois, A. Colletta, J. Wu, T. C. Major, C. Xi, R. H. Bartlett, A. J. Matzger, M. E. Meyerhoff, *ACS Appl. Mater. Interfaces* **2015**, *7*, 22218.
- [57] G. Pezzotti, *ACS Appl. Mater. Interfaces* **2019**, *11*, 26619.
- [58] G. Pezzotti, *Mater. Today Chem.* **2018**, *9*, 80.
- [59] B. S. Bal, M. N. Rahaman, *Acta Biomater.* **2012**, *8*, 2889.
- [60] M. N. Rahaman, A. Yao, B. S. Bal, J. P. Garino, M. D. Ries, *J. Am. Ceram. Soc.* **2007**, *90*, 1965.
- [61] G. Pezzotti, N. Oba, W. Zhu, E. Marin, A. Rondinella, F. Boschetto, B. McEntire, K. Yamamoto, B. S. Bal, *Acta Biomater.* **2017**, *64*, 411.
- [62] S. S. Lee, L. Laganenka, X. Du, W.-D. Hardt, S. J. Ferguson, *Front. Bioeng. Biotechnol.* **2021**, *9*, 794586.
- [63] G. Pezzotti, R. M. Bock, B. J. McEntire, E. Jones, M. Boffelli, W. Zhu, G. Baggio, F. Boschetto, L. Puppulin, T. Adachi, et al., *Langmuir* **2016**, *32*, 3024.
- [64] G. Pezzotti, F. Boschetto, E. Ohgitani, Y. Fujita, M. Shin-Ya, T. Adachi, T. Yamamoto, N. Kanamura, E. Marin, W. Zhu, et al., *Mater. Today Bio* **2021**, *12*, 100144.
- [65] R. B. Heimann, *Ceramics* **2021**, *4*, 208.
- [66] M. Rahaman, W. Xiao, *Int. J. Appl. Ceram. Technol.* **2018**, *15*, 861.
- [67] P. Gao, H. Qiu, K. Xiong, X. Li, Q. Tu, H. Wang, N. Lyu, X. Chen, N. Huang, Z. Yang, *Biomaterials* **2020**, *248*, 119981.
- [68] M. J. Neufeld, A. Lutzke, W. M. Jones, M. M. Reynolds, *ACS Appl. Mater. Interfaces* **2017**, *9*, 35628.

- [69] V. Wonoputri, C. Gunawan, S. Liu, N. Barraud, L. H. Yee, M. Lim, R. Amal, *ACS Appl. Mater. Interfaces* **2015**, *7*, 22148.
- [70] Q. Zhao, Y. Fan, Y. Zhang, J. Liu, W. Li, Y. Weng, *ACS Appl. Mater. Interfaces* **2019**, *11*, 7872.
- [71] V. Wonoputri, C. Gunawan, S. Liu, N. Barraud, L. H. Yee, M. Lim, R. Amal, *ACS Appl. Mater. Interfaces* **2016**, *8*, 30502.
- [72] X. Li, F. Shen, K. Wang, S. Lin, L. Zhou, S. Chen, J. Wang, N. Huang, *J. Mater. Chem. B* **2018**, *6*, 7582.
- [73] Q. Song, L. Li, K. Xiong, W. Tian, J. Lu, J. Wang, N. Huang, Q. Tu, Z. Yang, *Biomater. Sci.* **2019**, *7*, 3741.
- [74] Z. Yang, Y. Yang, K. Xiong, J. Wang, H. Lee, N. Huang, *Chem. Mater.* **2018**, *30*, 5220.
- [75] Y. Fan, X. Pan, K. Wang, S. Wu, H. Han, P. Yang, R. Luo, H. Wang, N. Huang, W. Tan, et al., *Colloids Surf. B* **2016**, *145*, 122.
- [76] B. Qu, L. Yuan, J. Li, J. Wang, H. Lv, X. Yang, *J. Mater. Chem. B* **2019**, *7*, 150.
- [77] D. Tang, S. Chen, D. Hou, J. Gao, L. Jiang, J. Shi, Q. Liang, D. Kong, S. Wang, *Mater Sci Eng C* **2018**, *84*, 1.
- [78] S. Jin, J. Huang, X. Chen, H. Gu, D. Li, A. Zhang, X. Liu, H. Chen, *ACS Appl. Bio Mater.* **2020**, *3*, 570.
- [79] P. Horcajada, R. Gref, T. Baati, P. K. Allan, G. Maurin, P. Couvreur, G. Férey, R. E. Morris, C. Serre, *Chem. Rev.* **2012**, *112*, 1232.
- [80] J. Chaudiere, O. Courtin, J. Leclaire, *Arch. Biochem. Biophys.* **1992**, *296*, 328.
- [81] Y. Hou, Z. Guo, J. Li, P. G. Wang, *Bioch. Biophys. Res. Commun.* **1996**, *228*, 88.
- [82] W. Cha, M. E. Meyerhoff, *Biomaterials* **2007**, *28*, 19.
- [83] B. Qu, L. Yuan, J. Li, J. Wang, H. Lv, X. Yang, *J. Mater. Chem. B* **2019**, *7*, 150.
- [84] T. Ren, Z. Mao, J. Guo, C. Gao, *Langmuir* **2013**, *29*, 6386.
- [85] H. Gu, X. Chen, Q. Yu, X. Liu, W. Zhan, H. Chen, J. L. Brash, *J. Mater. Chem. B* **2017**, *5*, 604.
- [86] M. Alagem-Shafir, E. Kivovich, I. Tzchori, N. Lanir, M. Falah, M. Y. Flugelman, U. Dinnar, R. Beyar, N. Lotan, S. S. Sivan, *Acta Biomater.* **2014**, *10*, 2304.
- [87] A. K. Winther, B. Fejerskov, M. ter Meer, N. B. S. Jensen, R. Dillion, J. E. Schaffer, R. Chandrawati, M. M. Stevens, L. J. Schultze Kool, U. Simonsen, et al., *ACS Appl. Mater.*

*Interfaces* **2018**, *10*, 10741.

- [88] B. Gunasekera, C. Abou Diwan, G. Altawallbeh, H. Kalil, S. Maher, S. Xu, M. Bayachou, *ACS Appl. Mater. Interfaces* **2018**, *10*, 7745.
- [89] M. Rath, I. Muller, P. Kropf, E. I. Closs, M. Munder, *Front. Immunol.* **2014**, *5*, 532
- [90] H. M. Abu-Soud, R. Gachhui, F. M. Raushel, D. J. Stuehr, *J. Biol. Chem.* **1997**, *272*, 17349.
- [91] B. C. Kone, T. Kunczewicz, W. Zhang, Z.-Y. Yu, *Renal Physiol.* **2003**, *285*, 13.
- [92] M. W. Vaughn, L. Kuo, J. C. Liao, *Am. J. Physiol.-Heart Circ.* **1998**, *274*, H2163.
- [93] J. Pant, M. J. Goudie, S. P. Hopkins, E. J. Brisbois, H. Handa, *ACS Appl. Mater. Interfaces* **2017**, *9*, 15254.
- [94] P. Singha, C. D. Workman, J. Pant, S. P. Hopkins, H. Handa, *J. Biomed. Mater. Res.* **2019**, *107*, 1425.
- [95] A. Mondal, M. Douglass, S. P. Hopkins, P. Singha, M. Tran, H. Handa, E. J. Brisbois, *ACS Appl. Mater. Interfaces* **2019**, *11*, 34652.
- [96] S. Huang, H. Liu, K. Liao, Q. Hu, R. Guo, K. Deng, *ACS Appl. Mater. Interfaces* **2020**, acsami.0c04080.
- [97] H. A. J. Hibbard, M. M. Reynolds, *ACS Appl. Bio Mater.* **2020**, *3*, 5367.
- [98] W. H. Lee, H. Ren, J. Wu, O. Novak, R. B. Brown, C. Xi, M. E. Meyerhoff, *ACS Biomater. Sci. Eng.* **2016**, *2*, 1432.
- [99] B. Tuesuwan, V. Vongsutilers, *J. Pharm. Sci.* **2021**, *110*, 3118.

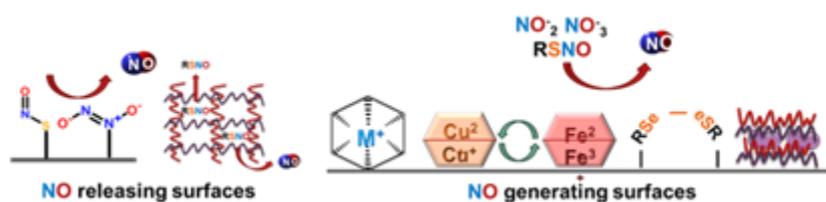
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This review summarizes the strategies described over the past 5 years to design nitric oxide (NO) delivering surfaces for cardiovascular or antibacterial purposes. An emphasis is given on three parameters: properties of the substrate, releasing and/or generating kinetics of NO and biocompatibility and stability of the surface. Their characteristics, advantages, limitations and applications regarding the chosen strategies are depicted.